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METASTATIC COLORECTAL CANCER VACCINE

FIELD OF THE INVENTION

The invention relates to prophylactic and therapeutic vaccines for protecting individuals against metastatic colorectal cancer and for treating individuals who are suffering from metastatic colorectal cancer.

BACKGROUND OF THE INVENTION

Colorectal cancer is the third most common neoplasm worldwide. The mortality rate of newly diagnosed large bowel cancer approaches 50% and there has been little improvement over the past 40 years. Most of this mortality reflects local, regional and distant metastases.

Surgery is the mainstay of treatment for colorectal cancer but recurrence is frequent. Colorectal cancer has proven resistant to chemotherapy, although limited success has been achieved using a combination of 5-fluorouracil and levamisole. Surgery has had the largest impact on survival and, in some patients with limited disease, achieves a cure. However, surgery removes bulk tumor, leaving behind microscopic residual disease which ultimately results in recrudescence.

Early detection of primary, metastatic, and recurrent disease can significantly impact the prognosis of individuals suffering from colorectal cancer. Large bowel cancer diagnosed at an early stage has a significantly better outcome than that diagnosed at more advanced stages. Similarly, diagnosis of

metastatic or recurrent disease earlier potentially carries with it a better prognosis.

Recent discoveries have shown that mutations of the human APC (Adenomatous Polyposis Coli) gene are responsible for both sporadic and familial colorectal cancers. Germ-line mutations of APC are found in inherited familial cancers such as Gardner's syndrome, attenuated adenomatous polyposis coli, heredity flat adenoma syndrome and familial adenomatous polyposis (FAP). FAP is an autosomal dominant inherited disease predisposing the patient to colon cancer. Patients inheriting a single mutant allele of APC develop hundreds to thousands of adenomatous polyps in the second to third decades of life, which if left untreated progress to malignant carcinomas. Genetic linkage analysis localized the APC gene to human chromosome 5q21-q22, a region frequently associated with allelic loss of the wildtype 5q allele. Mutations in APC are also implicated in sporadic colorectal cancers and in extracolonic tumors, such as gastric and small intestinal polyps, osteomas, sarcomas and desmoidal tumors.

There is a need for improved methods of treating individuals suffering from metastasized colon cancer. There is a need for compositions useful to treat individuals suffering from metastasized colon cancer. There is a need for improved methods of preventing a recurrence of metastasized colon cancer in individuals who have been treated for metastasized colon cancer. There is a need for compositions useful to prevent a recurrence of metastasized colon cancer in individuals who have been treated for metastasized colon cancer. There is a need for improved methods of preventing metastasized colon cancer in individuals, particularly those who have been identified as having a genetic predisposition for colon cancer. There is a need for compositions useful for preventing metastasized colon cancer in individuals.

SUMMARY OF THE INVENTION

The invention relates to an isolated protein comprising at least one epitope of human ST receptor protein.

In some embodiments, the epitope is an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the isolated protein comprises the human ST receptor protein. In some embodiments, the isolated protein consists of the human ST receptor protein.

The invention relates to vaccines which comprise such proteins and a pharmaceutically acceptable carrier or diluent.

The invention relates to a haptenized protein comprising at least one epitope of human ST receptor protein. In some embodiments, the epitope is an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the epitope is an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized protein comprises the human ST receptor protein. In some embodiments, the haptenized protein consists of the human ST receptor protein.

The invention relates to vaccines which comprise such haptenized proteins and a pharmaceutically acceptable carrier or diluent.

The invention relates to nucleic acid molecules that encode a protein comprising at least one epitope of human ST

receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes a protein that comprises the human ST receptor protein. In some embodiments, the nucleic acid molecule encodes human ST receptor protein. In some embodiments, the nucleic acid molecule is a plasmid.

The invention relates to vaccines which comprise such nucleic acid molecules and a pharmaceutically acceptable carrier or diluent.

The invention relates to vectors that comprise nucleic acid molecules that encode a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that protein comprises the extracellular domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that

encodes a protein that comprises the transmembrane domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that comprises the cytoplasmic domain of the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes a protein that comprises the human ST receptor protein. In some embodiments, the vector comprises a nucleic acid molecule that encodes human ST receptor protein. In some embodiments, the vector is a virus or a bacterial cell. In some embodiments, the vector is a recombinant vaccinia virus.

The invention relates to vaccines which comprise such vectors and a pharmaceutically acceptable carrier or diluent.

The invention relates to killed or inactivated cells or particles that comprise a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the extracellular domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise a protein with an epitope of the cytoplasmic domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the extracellular domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles vector comprise the transmembrane domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the cytoplasmic domain of the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles comprise the human ST receptor protein. In some embodiments, the killed or inactivated cells or particles vector is a killed or inactivated colorectal tumor cells.

The invention relates to vaccines which comprise such killed or inactivated cells or particles and a pharmaceutically acceptable carrier or diluent.

5 The invention relates to haptenized killed or inactivated cells or particles that comprise a protein comprising at least one epitope of human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the extracellular domain of the human ST receptor protein. In some
10 embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise a protein with an epitope of the cytoplasmic
15 domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the extracellular domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles vector comprise the
20 transmembrane domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the cytoplasmic domain of the human ST receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles comprise the human ST
25 receptor protein. In some embodiments, the haptenized killed or inactivated cells or particles vector is a killed or inactivated colorectal tumor cells.

The invention relates to vaccines which comprise such haptenized killed or inactivated cells or particles and a
30 pharmaceutically acceptable carrier or diluent.

The present invention relates to methods of treating individuals suffering from metastasized colorectal cancer. The method of the present invention provides administering to such an individual a therapeutically effective amount of a vaccine
35 of the invention. The invention relates to the use of such vaccines as immunotherapeutics.

The present invention relates to methods of treating individuals susceptible metastasized colorectal cancer. The method of the present invention provides administering to such an individual an amount of a vaccine of the invention effective
5 to prevent or combat metastasized colorectal cancer. The present invention relates to the use of the vaccines of the invention prophylactically.

DETAILED DESCRIPTION OF THE INVENTION

U.S. Serial Number 08/141,892 filed on October 26,
10 1993 (which is scheduled to issue on May 21, 1996 as U.S. Patent Number 5,518,888), U.S. Serial Number 08/305,056 filed on September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed October 26, 1994, which are each incorporated herein by reference, describe compositions for and
15 methods of treating, imaging and detecting metastasized colon cancer.

As used herein, the terms "ST receptor" and "guanylin cyclase C" are interchangeable and meant to refer to the receptors found on colorectal cells, including local and
20 metastasized colorectal cancer cells, which bind to ST. In normal individuals, ST receptors are found exclusively in cells of intestine, in particular in cells in the duodenum, small intestine (jejunum and ileum), the large intestine, colon (cecum, ascending colon, transverse colon, descending colon and
25 sigmoid colon) and rectum.

As used herein, the term "colorectal cancer" is meant to include the well-accepted medical definition that defines colorectal cancer as a medical condition characterized by cancer of cells of the intestinal tract below the small
30 intestine (i.e. the large intestine (colon), including the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and rectum). Additionally, as used herein, the term "colorectal cancer" is meant to further include medical conditions which are characterized by cancer of cells of the
35 duodenum and small intestine (jejunum and ileum). The definition of colorectal cancer used herein is more expansive

than the common medical definition but is provided as such since the cells of the duodenum and small intestine also contain ST receptors and are therefore amenable to the methods of the present invention using the compounds of the present invention.

As used herein, the term "metastasis" is meant to refer to the process in which cancer cells originating in one organ or part of the body relocate to another part of the body and continue to replicate. Metastasized cells subsequently form tumors which may further metastasize. Metastasis thus refers to the spread of cancer from the part of the body where it originally occurs to other parts of the body. The present invention relates to methods of delivering active agents to metastasized colorectal cancer cells.

As used herein, the term "metastasized colorectal cancer cells" is meant to refer to colorectal cancer cells which have metastasized; colorectal cancer cells localized in a part of the body other than the duodenum, small intestine (jejunum and ileum), large intestine (colon), including the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, and rectum.

As used herein, "an individual is suspected of being susceptible to metastasized colorectal cancer" is meant to refer to an individual who is at an above-average risk of developing metastasized colorectal cancer. Examples of individuals at a particular risk of developing metastasized colorectal cancer are those whose family medical history indicates above average incidence of colorectal cancer among family members and/or those who have already developed colorectal cancer and have been effectively treated who therefore face a risk of relapse and recurrence. Other factors which may contribute to an above-average risk of developing metastasized colorectal cancer which would thereby lead to the classification of an individual as being suspected of being susceptible to metastasized colorectal cancer may be based upon an individual's specific genetic, medical and/or behavioral background and characteristics.

Heat stable toxin ST, which is produced by *E. coli*, as well as other organisms, is responsible for endemic diarrhea in developing countries and travelers diarrhea. ST induces intestinal secretion by binding to specific receptors, ST
5 receptors, in the apical brush border membranes of the mucosal cells lining the intestinal tract. Binding of ST to ST receptors is non-covalent and occurs in a concentration-dependent and saturable fashion. Once bound, ST-ST receptor complexes appear to be internalized by intestinal cells, i.e.
10 transported from the surface into the interior of the cell. Binding of ST to ST receptors triggers a cascade of biochemical reactions in the apical membrane of these cells resulting in the production of a signal which induces intestinal cells to secrete fluids and electrolytes, resulting in diarrhea.

15 ST receptors are unique in that they are only localized in the apical brush border membranes of the cells lining the intestinal tract. Indeed, they are not found in any other cell type in placental mammals. In addition, ST
20 receptors are almost exclusively localized to the apical membranes, with little being found in the basolateral membranes on the sides of intestinal cells.

Mucosal cells lining the intestine are joined together by tight junctions which form a barrier against the
25 passage of intestinal contents into the blood stream and components of the blood stream into the intestinal lumen. Therefore, the apical location of ST receptors isolates these receptors from the circulatory system so that they may be considered to exist separate from the rest of the body;
30 essentially the "outside" of the body. Therefore, the rest of the body is considered "outside" the intestinal tract, i.e. extraintestinal. Compositions administered "outside" the intestinal tract are maintained apart and segregated from the only cells which normally express ST receptors. Conversely,
35 tissue samples taken from tissue outside of the intestinal tract, i.e. extraintestinal tissue samples, do not normally contain cells which express ST receptors.

In individuals suffering from colorectal cancer, the cancer cells are often derived from cells that produce and display the ST receptor and these cancer cells continue to produce and display the ST receptor on their cell surfaces. Indeed, T84 cells, which are human colonic adenocarcinoma cells isolated from lung metastases, express ST receptors on their cell surface. Similarly, HT29glu-cells, which are human colonic adenocarcinoma cells, express receptors for ST. Thus, in individuals suffering from colorectal cancer, some metastasized intestinal cancer cells express ST receptors.

An effort was undertaken to determine the proportion of colorectal tumors which have the ST receptor. Each of the tumors tested were independently confirmed to be colorectal cancer by standard techniques of surgical pathology. Every one of the colorectal cancer tumors tested, including local colorectal tumors and metastasized colorectal tumors (liver, lung, lymph node, peritoneum, ovary) possessed ST receptors. In each case, the affinity and density of receptors was amenable for targeting. Normal liver, lymph node, peritoneum, gall bladder, ovary, stomach, kidney and lung cells were found not to possess ST receptors.

When such cancer cells metastasize, the metastasized cancer cells continue to produce and display the ST receptor. The expression of ST receptors on the surfaces of metastatic tumors provides a target which can be used to distinguish the metastasized colorectal cancer cells from normal extraintestinal cells. This target is useful in the detection, imaging and treatment of metastasized colorectal cancer.

According to the present invention, the ST receptor protein serves as a target against which a protective and therapeutic immune response can be induced. Specifically, vaccines are provided which induce an immune response against the ST receptor protein. The vaccines of the invention include, but are not limited to, the following vaccine technologies:

- 1) DNA vaccines, i.e. vaccines in which DNA that encodes at least an epitope from ST receptor protein is

administered to an individual's cells where the epitope is expressed and serves as a target for an immune response;

2) infectious vector mediated vaccines such as recombinant adenovirus, vaccinia, *Salmonella*, and BCG wherein
5 the vector carries genetic information that encodes at least an epitope of ST receptor protein such that when the infectious vector is administered to an individual, the epitope is expressed and serves as a target for an immune response;

3) killed or inactivated vaccines which a) comprise
10 either killed cells or inactivated viral particles that display at least an epitope of the ST receptor protein and b) when administered to an individual serves as a target for an immune response;

3) haptenized killed or inactivated vaccines which
15 a) comprise either killed cells or inactivated viral particles that display at least an epitope of the ST receptor, b) are haptenized to be more immunogenic and c) when administered to an individual serves as a target for an immune response;

4) subunit vaccines which are vaccines that include
20 protein molecules that include at least an epitope the ST receptor protein; and

5) haptenized subunit vaccines which are vaccines that a) include protein molecules that include at least an epitope the ST receptor protein and b) are haptenized to be
25 more immunogenic.

The present invention relates to administering to an individual a protein or nucleic acid molecule that comprises or encodes, respectively, an immunogenic epitope against which an therapeutic and prophylactic immune response can be induced.
30 Such epitopes are generally at least 6-8 amino acids in length. The vaccines of the invention therefore comprise proteins which are at least, or nucleic acids which encode at least, 6-8 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic
35 acids which encode at least, the entire ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least 10 to about

1000 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 25 to about 500 amino acids in length from ST receptor protein. The
5 vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 50 to about 400 amino acids in length from ST receptor protein. The vaccines of the invention may comprise proteins which are at least, or nucleic acids which encode at least, about 100 to
10 about 300 amino acids in length from ST receptor protein. In preferred embodiments, fragments of ST receptor protein that include the extracellular domain are provided.

The present invention relates to compositions for and methods of treating individuals who are known to have
15 metastasized colorectal cancer. Metastasized colorectal cancer may be diagnosed by those having ordinary skill in the art using art accepted clinical and laboratory pathology protocols and/or those described in U.S. Serial Number 08/141,892 filed on October 26, 1993, U.S. Serial Number 08/305,056 filed on
20 September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed October 26, 1994. The present invention provides an immunotherapeutic vaccine useful to treat individuals who have been diagnosed as suffering from metastasized colorectal cancer. The immunotherapeutic vaccines
25 of the present invention may be administered in combination with other therapies including, but not limited to those described in U.S. Serial Number 08/141,892 filed on October 26, 1993, U.S. Serial Number 08/305,056 filed on September 13, 1994, and PCT Application Serial Number PCT/US94/12232 filed
30 October 26, 1994.

The present invention relates to compositions for and methods of preventing metastatic colorectal cancer in individual is suspected of being susceptible to metastasized colorectal cancer. Such individuals include those whose
35 family medical history indicates above average incidence of colorectal cancer among family members and/or those who have already developed colorectal cancer and have been effectively

treated who therefore face a risk of relapse and recurrence. Such individuals include those which have been diagnosed as having colorectal cancer including localized only or localized and metastasized colorectal cancer which has been resected or
5 otherwise treated. Such individuals also include those with an elevated risk as ascertained by genetic evaluation. For example, individuals with APC mutations can be identified following the U.S. Patent Number 5,352,775 issued October 4, 1992 to Albertsen et al., which is incorporated herein by
10 reference. Furthermore, such individuals include: those suffering from inflammatory bowel disease, particularly those with ulcerative colitis; those with colonic polyps; those with familial adenomatous polyposis, a heritable mutation predisposing patients to develop large numbers of intestinal
15 polyps; those with Peutz-Jeghers syndrome; those with hereditary nonpolyposis coli, a heritable mutation which predisposes people to develop colon carcinoma; those with Turcot syndrome-colon carcinoma in conjunction with independent tumors of the central nervous system; and individuals engaging
20 in rectal intercourse. The vaccines of the present invention may be to susceptible individuals prophylactically to prevent and combat colorectal cancer metastasis.

The invention relates to compositions which are the active components of such vaccines or required to make the
25 active components, to methods of making such compositions including the active components, and to methods of making and using vaccines.

The nucleotide sequence that encodes human ST receptor protein is disclosed as SEQ ID NO:1. The amino acid
30 sequence of human ST receptor is also disclosed in SEQ ID NO:1. Generally, the extracellular domain refers to the amino acids about 24 to about 454. The transmembrane region refers to amino acids about 455 to about 475. The cytoplasmic domain refers to amino acids about 476 to about 1093.

35 Accordingly, some aspects of the invention relate to isolated proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular

domain, transmembrane domain or cytoplasmic domain. In preferred embodiments, the protein comprises at least one epitope from the extracellular domain. The protein may comprise ST receptor protein sequences or consist of ST
5 receptor protein sequences. The protein may comprise the entire ST receptor protein, consist of the entire ST receptor protein, comprise a fragment of the ST receptor protein, or consist of a fragment of the ST receptor protein. In some preferred embodiments, the protein is a soluble form of the
10 extracellular domain. In some preferred embodiments, the protein is a soluble form of the extracellular domain with a portion of the transmembrane domain.

Some aspects of the invention relate to the above described isolated proteins which are hapttenized to render them
15 more immunogenic. That is, some aspects of the invention relate to hapttenized proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular domain, transmembrane domain or cytoplasmic domain. The protein may comprise ST receptor protein sequences
20 or consist of ST receptor protein sequences. The protein may comprise the entire ST receptor protein, consist of the entire ST receptor protein, comprise a fragment of the ST receptor protein, or consist of a fragment of the ST receptor protein. In some preferred embodiments, the hapttenized protein comprises
25 a soluble form of the extracellular domain. In some preferred embodiments, the hapttenized protein is a soluble form of the extracellular domain with a portion of the transmembrane domain.

Some aspects of the invention nucleic acid molecules
30 that encode the above described isolated proteins.

Accordingly, some aspects of the invention relate to isolated nucleic acid molecules that encode proteins that comprise at least one ST receptor epitope. The epitope may be from the ST receptor extracellular domain, transmembrane domain
35 or cytoplasmic domain. In preferred embodiments, the isolated nucleic acid molecules encodes a protein that comprises at least one epitope from the extracellular domain. The isolated

nucleic acid molecule may encode a protein that comprises or consists of ST receptor protein sequences. The isolated nucleic acid molecule may encode a protein that comprises or consists of the entire ST receptor protein, or a protein that
5 comprises or consists of a fragment of the ST receptor protein. In some embodiments, the isolated nucleic acid molecule encodes non-ST receptor protein sequences which are useful to render the ST receptor protein sequences more immunogenic.

Naked DNA vaccines are described in PCT/US90/01515,
10 which is incorporated herein by reference. Others teach the use of liposome mediated DNA transfer, DNA delivery using microprojectiles (U.S. Patent No. 4,945,050 issued July 31, 1990 to Sanford et al., which is incorporated herein by reference), and DNA delivery using electroporation. In each
15 case, the DNA may be plasmid DNA that is produced in bacteria, isolated and administered to the animal to be treated. The plasmid DNA molecules are taken up by the cells of the animal where the sequences that encode the protein of interest are expressed. The protein thus produced provides a therapeutic
20 or prophylactic effect on the animal.

The use of vectors including viral vectors and other means of delivering nucleic acid molecules to cells of an individual in order to produce a therapeutic and/or prophylactic immunological effect on the individual are
25 similarly well known. Recombinant vaccines that employ vaccinia vectors are, for example, disclosed in U.S. Patent Number 5,017,487 issued May 21, 1991 to Stunnenberg et al. which is incorporated herein by reference.

In some cases, tumor cells from the patient are
30 killed or inactivated and administered as a vaccine product. Berd et al. May 1986 *Cancer Research* 46:2572-2577 and Berd et al. May 1991 *Cancer Research* 51:2731-2734, which are incorporated herein by reference, describes the preparation and use of tumor cell based vaccine products. According to some
35 aspects of the present invention, the methods and techniques described in Berd et al. are adapted by using colorectal cancer cells instead of melanoma cells.

The manufacture and use of subunit vaccines are well known. One having ordinary skill in the art can isolate the nucleic acid molecule that encode ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. Once isolated, the nucleic acid molecule can be inserted it into an expression vector using standard techniques and readily available starting materials. Rudner et al. May 1995 *Proc. Natl. Acad. Sci. USA* 92:5169-5173 disclosed the cloning and expression of the extracellular domain of human ST receptor and purification of the same using a Flag immunoaffinity epitope and antibody therefor.

The recombinant expression vector that comprises a nucleotide sequence that encodes the nucleic acid molecule that encode ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. As used herein, the term "recombinant expression vector" is meant to refer to a plasmid, phage, viral particle or other vector which, when introduced into an appropriate host, contains the necessary genetic elements to direct expression of the coding sequence that encodes the protein. The coding sequence is operably linked to the necessary regulatory sequences. Expression vectors are well known and readily available. Examples of expression vectors include plasmids, phages, viral vectors and other nucleic acid molecules or nucleic acid molecule containing vehicles useful to transform host cells and facilitate expression of coding sequences. The recombinant expression vectors of the invention are useful for transforming hosts to prepare recombinant expression systems for preparing the isolated proteins of the invention.

The present invention relates to a host cell that comprises the recombinant expression vector that includes a nucleotide sequence that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. Host cells for use in well known recombinant expression systems for production of proteins are well known and readily available. Examples of host cells include bacteria cells such as *E. coli*, yeast cells such as *S.*

cerevisiae, insect cells such as *S. frugiperda*, non-human mammalian tissue culture cells chinese hamster ovary (CHO) cells and human tissue culture cells such as HeLa cells.

The present invention relates to a transgenic non-human mammal that comprises the recombinant expression vector that comprises a nucleic acid sequence that encodes the proteins of the invention. Transgenic non-human mammals useful to produce recombinant proteins are well known as are the expression vectors necessary and the techniques for generating transgenic animals. Generally, the transgenic animal comprises a recombinant expression vector in which the nucleotide sequence that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof operably linked to a mammary cell specific promoter whereby the coding sequence is only expressed in mammary cells and the recombinant protein so expressed is recovered from the animal's milk.

In some embodiments, for example, one having ordinary skill in the art can, using well known techniques, insert such DNA molecules into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for production of collagen in *E. coli*. The commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may, for example, be used for production in *S. cerevisiae* strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, CA) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I (Invitrogen, San Diego, CA) may, for example, be used for production in mammalian cells such as Chinese Hamster Ovary cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof using routine techniques and readily available starting materials. (See e.g., Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold

Spring Harbor Press (1989) which is incorporated herein by reference.) Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

5 One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and
10 polyadenylation signals, and preferably enhancers, are readily available and known in the art for a variety of hosts. See e.g., Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989).

 A wide variety of eukaryotic hosts are also now
15 available for production of recombinant foreign proteins. As in bacteria, eukaryotic hosts may be transformed with expression systems which produce the desired protein directly, but more commonly signal sequences are provided to effect the secretion of the protein. Eukaryotic systems have the
20 additional advantage that they are able to process introns which may occur in the genomic sequences encoding proteins of higher organisms. Eukaryotic systems also provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or
25 derivatization of certain amino acid residues, conformational control, and so forth.

 Commonly used eukaryotic systems include, but is not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. Suitable promoters
30 are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, e.g. the baculovirus polyhedron promoter. As above, promoters can be either constitutive or inducible. For example, in mammalian systems, the mouse metallothionein
35 promoter can be induced by the addition of heavy metal ions.

 The particulars for the construction of expression systems suitable for desired hosts are known to those in the

art. Briefly, for recombinant production of the protein, the DNA encoding the polypeptide is suitably ligated into the expression vector of choice. The DNA is operably linked to all regulatory elements which are necessary for expression of the DNA in the selected host. One having ordinary skill in the art can, using well known techniques, prepare expression vectors for recombinant production of the polypeptide.

The expression vector including the DNA that encodes the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof is used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign DNA takes place. The protein of the present invention thus produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art. One having ordinary skill in the art can, using well known techniques, the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof that is produced using such expression systems. The methods of purifying the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof using antibodies which specifically bind to the protein are well known. Antibodies which specifically bind to a particular protein may be used to purify the protein from natural sources using well known techniques and readily available starting materials. Such antibodies may also be used to purify the protein from material present when producing the protein by recombinant DNA methodology. The present invention relates to antibodies that bind to an epitope which is present on the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab fragments and F(ab)₂ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies. Antibodies that bind to an epitope which

is present on the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof are useful to isolate and purify the protein from both natural sources or recombinant expression systems using well known techniques such as affinity chromatography. Immunoaffinity techniques generally are described in Waldman et al. 1991 *Methods of Enzymol.* 195:391-396, which is incorporated herein by reference. Antibodies are useful to detect the presence of such protein in a sample and to determine if cells are expressing the protein. The production of antibodies and the protein structures of complete, intact antibodies, Fab fragments and F(ab)₂ fragments and the organization of the genetic sequences that encode such molecules are well known and are described, for example, in Harlow, E. and D. Lane (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. which is incorporated herein by reference. Briefly, for example, the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, or an immunogenic fragment thereof is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, the hybridoma which produces them is cultured to produce a continuous supply of antibodies.

In some embodiments of the invention, transgenic non-human animals are generated. The transgenic animals according to the invention contain nucleotides that encode the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof under the regulatory control of a mammary specific promoter. One having ordinary skill in the art using standard techniques, such as those taught in U.S. Patent No. 4,873,191 issued October 10, 1989 to Wagner and U.S. Patent No. 4,736,866 issued

April 12, 1988 to Leder, both of which are incorporated herein by reference, can produce transgenic animals which produce the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof.
5 Preferred animals are goats and rodents, particularly rats and mice.

In addition to producing these proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce the ST receptor protein or a fragment
10 thereof or a protein that comprises the ST receptor protein or a fragment thereof of the invention. Such techniques are well known to those having ordinary skill in the art and are useful if derivatives which have substitutions not provided for in DNA-encoded protein production.

15 In some embodiments, the protein that makes up a subunit vaccine or the cells or particles of a killed or inactivated vaccine may be haptenized to increase immunogenicity. In some cases, the haptenization is the conjugation of a larger molecular structure to the ST receptor
20 protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof. In some cases, tumor cells from the patient are killed and haptenized as a means to make an effective vaccine product. In cases in which other cells, such as bacteria or eukaryotic cells which are
25 provided with the genetic information to make and display the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, are killed and used as the active vaccine component, such cells are haptenized to increase immunogenicity. Haptenization is well
30 known and can be readily performed.

Methods of haptenizing cells generally and tumor cells in particular are described in Berd et al. May 1986 *Cancer Research* 46:2572-2577 and Berd et al. May 1991 *Cancer Research* 51:2731-2734, which are incorporated herein by
35 reference. Additional haptenization protocols are disclosed in Miller et al. 1976 *J. Immunol.* 117(5:1):1591-1526.

Haptenization compositions and methods which may be adapted to be used to prepare haptenized ST immunogens according to the present invention include those described in the following U.S. Patents which are each incorporated herein by reference: U.S. Patent Number 5,037,645 issued August 6, 1991 to Strahilevitz; U.S. Patent Number 5,112,606 issued May 12, 1992 to Shiosaka et al.; U.S. Patent Number 4,526,716 issued July 2, 1985 to Stevens; U.S. Patent Number 4,329,281 issued May 11, 1982 to Christenson et al.; and U.S. Patent Number 4,022,878 issued May 10, 1977 to Gross. Peptide vaccines and methods of enhancing immunogenicity of peptides which may be adapted to modify ST immunogens of the invention are also described in Francis et al. 1989 *Methods of Enzymol.* 178:659-676, which is incorporated herein by reference. Sad et al. 1992 *Immunology* 76:599-603, which is incorporated herein by reference, teaches methods of making immunotherapeutic vaccines by conjugating gonadotropin releasing hormone to diphtheria toxoid. ST immunogens may be similarly conjugated to produce an immunotherapeutic vaccine of the present invention. MacLean et al. 1993 *Cancer Immunol. Immunother.* 36:215-222, which is incorporated herein by reference, describes conjugation methodologies for producing immunotherapeutic vaccines which may be adaptable to produce an immunotherapeutic vaccine of the present invention. The hapten is keyhole limpet hemocyanin which may be conjugated to an ST immunogen.

As used herein, the term "ST receptor immunogen" is meant to refer to the ST receptor protein or a fragment thereof or a protein that comprises the ST receptor protein or a fragment thereof, haptenized ST receptor protein or a haptenized fragment thereof or a haptenized protein that comprises the ST receptor protein or a haptenized fragment thereof, cells and particles which display at least one ST receptor epitope, and haptenized cells and haptenized particles which display at least one ST receptor epitope

Vaccines according to some aspects of the invention comprise a pharmaceutically acceptable carrier in combination with an ST receptor immunogen. Pharmaceutical formulations are

well known and pharmaceutical compositions comprising such proteins may be routinely formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference. The present invention relates to an injectable pharmaceutical composition that comprises a pharmaceutically acceptable carrier and an ST receptor immunogen. The ST receptor immunogen is preferably sterile and combined with a sterile pharmaceutical carrier.

In some embodiments, for example, the ST receptor immunogen can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

An injectable composition may comprise the ST receptor immunogen in a diluting agent such as, for example, sterile water, electrolytes/dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and polyethylene glycol. The injectable must be sterile and free of pyrogens.

The vaccines of the present invention may be administered by any means that enables the immunogenic agent to be presented to the body's immune system for recognition and induction of an immunogenic response. Pharmaceutical compositions may be administered parenterally, i.e., intravenous, subcutaneous, intramuscular.

Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind

of concurrent treatment, frequency of treatment, and the effect desired. An amount of immunogen is delivered to induce a protective or therapeutically effective immune response. Those having ordinary skill in the art can readily determine the
5 range and optimal dosage by routine methods.